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Microbial biomass: An economical alternative for removal of heavy metals from waste water and its role in sustainable agriculture

Mahima Chaurasia[1], Sanjeev Kumar Srivastava[2*], Siddhartha Shukla[2]
[1] Assistant professor, Applied Sciences and Humanities, University: Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India
[2] Department of Environmental sciences, Dr. R. L. Avadh University, Ayodhya, Uttar Pradesh, India

Abstract
Heavy metal contamination due to natural and anthropogenic sources is a global environmental concern. Today indiscriminate and uncontrolled discharge of metal contaminated industrial effluents into the environment has become an issue of major concern. Heavy metals, being non-biodegradable and persistent, beyond a permissible concentration from unspecific compounds inside the cells thereby causing cellular toxicity. Release of heavy metal without proper treatment poses a significant threat to public health because of its persistence, bio magnifications and accumulation in food chain. Non-biodegradability and sludge production are the two major constraints of metal treatment. Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and ecofriendly nature. Recent advances have been made in understanding metal-microbe interaction and their application for metal accumulation / detoxification. The availability of variety of microbial biomass and their metal binding potential makes it a economical and sustainable option for developing effluent treatment process for removal and recovery of heavy metals.

Keywords: bioremediation techniques, heavy metal contamination, biosorption, microbial biomass, metal binding potential

Introduction

Contamination of heavy metals in the environment is a major global concern because of their toxicity and threat to human life and environment (Ceribasi and Yetis, 2001; Hernandez *et al.*, 1998) [4, 8]. Much research has been conducted on heavy metal contamination in soils from various anthropogenic sources such as industrial wastes, automobile emissions, mining activity and agricultural practices (Mudakavi and Narayana, 1997; Tyagi *et al.*, 1999) [11, 20]. The groups of heavy metals are about 65 and are defined in a number of criteria such as their cationic-hydroxide formation, hard-soft acids and bases, and more recently association with eutrophication and environmental toxicity.

Roane and Pepper (2000) [16] classified metals into three classes on the basis on the basis of their biological functions and effects: (1) the essential metals with known biological functions, (Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo and W), (2) the toxic metals (Ag, Cd, Sn, Au, Hg, Ti, Pb, Al, and metalloids Ge, As, Sb and Se) and (3) the nonessential, non-toxic metals with no known biological effects (Rb, Cs, Sr and T). Based on primary accumulation mechanisms in sediments, heavy metals are classified into five categories. (1) adsorptive and exchangeable, (2) bound to carbonate phases, (3) bound to reducible phases (Fe and Mn oxides), (4) bound to organic matter and sulfides, and (5) detrital or lattice metals (Salomons and Forstner, 1998) [18]. A sudden boost in the industrial activities has contributed quantitatively as well as qualitatively to the alarming increase in the discharge of metal pollutants into environmental sink, especially the aqueous environment. Dispersion of the metal ions in water bodies leads to their biomagnification through the food chain and results in increased toxicity. This fact renders the removal of heavy metals from aqueous solutions indispensible. Thus, the boon of affluence has in turn given rise to the curse of effluent. More than fifty percent of the heavy metal pollution has been accounted to the anthropogenic activities. The tragic episodes of 'Itai-Itai' and 'Minamata' brought into focus the global concern regarding the impact of environmental pollution on human health. Since ten several centers all over the world have been engaged in the development of processes for removal of heavy metals (Gupta and Mohapatra, 2003) [7].

The conventional processes used for effluent treatment are precipitation as hydroxides / sulphides, oxidation / reduction, and ion exchange. These are expensive especially when the contaminant metals are dissolved in large volumes and appear in the concentration range 10-100 ppm. Moreover these are not ecofriendly in nature and result in the production of large amount of sludge. As a result an aquatic problem is transformed into a solid waste disposal problem. Therefore, amongst the chemical absorbent only ion exchange resins were considered as the option for remediation with least ecological problems. However, chemical resins are expensive and the increasing demand for ecofriendly technologies has led to the search of low cost alternatives that could be considered as single use materials. In this light, biological materials have emerged as an ecofriendly and economic option. For a long time activated carbon and peat occupied the place of prominence among biosorbent's but since they were geographically restricted in distribution microbial biomass became the unanimous choice (Gupta and Mohapatra, 2003) [7].

Microbial biomass can bind heavy metals either actively or passively or by a combination of both processes. The passive phenomenon of 'biosorption' has several advantages over the active phenomenon of 'bioaccumulation'. Growth physiology of an organism varies drastically with variations in the effluent compositions making it difficult to express mathematically. On the other hand, biosorption involves use of non-growing biomass / dead biomass for sequestration of the metals, thus the process is independent of metabolic activity. Another major disadvantages of bioaccumulation is recovery of the accumulated metal by destructive means whereas in biosorption desorption is accomplished by simple physical methods without damaging the biosorbent's structural integrity. Moreover, biosorption has an edge over bioaccumulation with easy and cost effective procurement of the biomass either as a byproduct of a large scale fermentation process or bulk procurement from natural water bodies (Costa and Leite, 1990; Karna *et al.*, 1996; Volesky and Holan, 1995) [5, 10, 21].

Microbial Biomass: Taxonomic Considerations

A wide variety of biological materials have been exploited for their metal biosorption capacities. One can begin the count down from the classical use of activated carbon to peat and end up with the widespread reports of microbial biosorbent materials. Fungi, yeast and bacteria as byproducts of industrial fermentation processes while macro algae due to bulk availability of their biomasses from natural water bodies have attracted most of the attention as is evident from the literature (Senthil *et* al., 2000; Arica *et al.*, 2001 and Zakharova *et al.*, 2001) [19, 1, 22].

Fungi and yeasts belonging to the genera *Rhizopus, Aspergillus, Penicillium* and *Saccharomyces* have shown excellent metal biosorption capacities. Among bacteria, *Bacillus subtilis* has been identified to have potential for metal sequestration and has been used in the commercial biosorbent preparation. Though available as a cheap industrial by product, one major disadvantage of using biomasses from industrial processes is the presence of impurities due to the adhering fermentation broth residue that might interfere with its metal sorption capacity (Kapoor and Viraraghavan, 1995; Muraleedharan *et al.*, 1995; Sahoo *et al.*, 1992; Puranik and Paknikar, 1999) [9, 17, 15]. Dias *et al.*, 2000 have compared the biosorptive potential of waste biomasses obtained from four different distilleries. They observed marked variation in metal binding capacities of these biomasses. These variations could be attributed to diversity in the micro flora composition and culture conditions in each of these production media.

Developing Bio Sor bents for commercial application Immobilization and Reinforcement

Despite the tremendous potential of the algal cells for heavy metal removal they still face major challenges for being exploited commercially. Commercial biosorbents need to fulfill a number of criteria such as: (i) high biosorption capacity at equilibrium *i.e.* they should contain as little as possible of inert material in their binding sites (ii) favorable biosorption kinetics *i.e.* particles should be hydrophilic and porous in nature (iii) maintenance of smooth flow dynamics in a reactor, this prevents the use of either very small or strongly swelling particles in the column (iv) amenable to regeneration, this necessitates desorption by minimal possible volume of desorbing agent without damaging the biosorbent (v) good mechanical strength (vi) temperature stability and (vii) resistance to chemicals (Dias *et al.*, 2000) [6].

The tendency of the free algal cells to clump together leads to clogging of the column. This also necessitates excessive hydrostatic pressure to generate suitable flow rates. Moreover, the fragile nature of the free algal cells renders them susceptible to disintegration due to high pressures. All these limitations make it imperative to immobilize these into suitable matrices prior to their use as commercial biosorbents in columns. Not all algae require immobilization; macro algae such as *Sargassum* or *Chara* need only proper sizing prior to use while all micro algae require some degree of immobilization or pretreatment or hardening. Immobilization of the non-viable cells has been carried out in several matrices natural as well as synthetic (Gupta and Mohapatra, 2003) [7].

Apart from immobilization, reinforcement and cross linking also provides a suitable means of stabilizing the biosorbent. Cross linking can be achieved by formaldehyde, gluteraldehyde and / or polyethylene imine. Higher durability was reported for cross linked cells (triethylene tetramine and glutamic dialdehyde 1% w/v) of *Zoogloea ramigera.* The authors have reported the cross linked capsules to maintain their mechanical strength and adsorption / desorption capacity even after 30 cycles of repeated use (Ashkenazy *et al.*, 1999) [2].

Mechanism of Biosorption

Sound understanding of the chemical nature of the metal ions and the binding sites is imperative and indispensible to develop an understanding towards the mechanism of biosorption. On the other hand, complex nature of the biopolymers poses a hindrance in understanding the mechanism of the biosorption. Preliminary studies carried out on biosorption reveals it to be a complex interplay of the properties of the biomolecules of the cell wall and the chemical nature of the metal ion in question. Each of the microbial group is characterized by a distinct cell wall structure. Biosorption by these microbes is attributed mainly to the ligands present in the biomolecules of their cell wall polymers.

Binding Sites

The cell wall polymers provide a multitude of chemical groups such as hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate and phosphodiester. These chemical groups of the biopolymers in turn harbor the binding sites, which provide the ligand atoms to form complexes with metal ions. Each of the binding sites can participate in different binding mechanisms such as complexation and electrostatic attraction of metal cations. Consequently several mechanisms act in combination. In general for metal binding we can distinguish between ion exchange, sorption of electrically neutral material (soluble metal ligand complexes) to specific binding sites and micro precipitation. These main mechanisms are based on sorbate solvent interactions, which in turn rely on some combination of covalent, electrostatic and Vander Waal's forces.

Importance of the given group for biosorption of certain metal by Certain biomass depends on various factors such as (i) quantity of sites in the biosorbent material (ii) chemical state of the site (*i.e.* its availability) (iii) accessibility of the site (iv) affinity between the site and the metal (*i.e.* binding strength). For covalent metal binding even an already occupied site is theoretically available. But to what extent this site this site can be used by the metal ion for binding depends on the binding strength and concentration of the metal in comparison to that already occupying the site. For electrostatic binding a site is available only if it is ionized

Metal affinity towards biomolecules

The affinity of various metals varies with respect to the bimolecular ligand. The bond character in biosorption can be explained partially by Pearson's concept of hard and soft metallic ions (Pearson, 1969) [13]. This scale is based on the binding strength of the ions with F^- and I^-. Those metallic ions (e.g. Na, Mg, K, Ca etc.) which form strong binding with F^- are referred to as 'hard' while those forming relatively weaker bonds (e.g. Au, Ag, Pb, Hg, etc.) are referred to as 'soft' ions. Avery and Tobin, 1993 have studied the applicability of 'hard and soft' principle in predicting m etal sorption by *Saccharomyces cerevisiae.* There also exists a class of ions with intermediate degree of hardness (e.g. Zn, Cu, Co, Ni, Fe, etc.) and are referred to as 'transition' metals. The hard ions also serve, as essential biological nutrients while the soft ions are usually toxic in nature. The transition metals of intermediate hardness are less toxic and are present in certain biomolecules where they assist in mediating specific biochemical reactions. Among the ligand atoms 'O' and 'F' are considered hard, 'S and P' are considered soft while 'N' stays in the intermediate category.

Elution and Recovery of Metal

There are generally two fates of the metal laden biomass; either it is 'ashed off' (by incineration) or regenerated (by elution). The first alternative is preferred where the biosorbent material is supplied as a waste biomass, cheap and abundant. This reduces the volume of waste generated. The more desirable and economic option of regeneration is based on the selective stripping off the metal laden on the biomass by the use of desorbing agents. The desorbing agents or eluting solutions function by uncoupling the bonds formed between the metal and the biosorbent. Small quantity of the eluant results in high metal concentration in the resulting solution, thus making it amenable to easy and economical extraction procedures. Thus the solid / liquid ratio (S/L) is often used to express the efficiency of the eluant (Gupta and Mohapatra, 2003; Zakharova *et al.*, 2001) [7, 22].

Chelating agents, salts and alkali solutions proved to be the best eluant while mineral acids though elute considerable amount of metal, usually cause damage to the biosorbent. This in turn affects the second resorption and subsequent resorption cycle. Philip *et al.*, 2000 have reported 95% desorption of the loaded U from *Pseudomonas aeruginosa* using 0.2 M HCL. They however observed an adverse effect of the acid on the viability of the cells. This drawback resulted in opting for citrate buffer (0.2M, *p*H 4.0) as the eluant of choice with 80% desorption. Similar decrease in biosorption capacity of beads has been reported for elution of metal laden beads of *Citrobacter* biomass by 0.1 M HCl (Puranik and Paknikar, 1999) [15].

Conclusions

Biosorption is an economically feasible; technically efficient technology for metal removal / recovery and can comfortably fit into the metal treatment processes and is eco-friendly in nature. Inspite, of these advantages why the biosorption / wastewater treatment has remained as an embryonic industry? This is because not all the companies, which generate metal polluted wastewater, will have the capability or the interest to do anything other than the basic treatment to comply with the legislative. Hence, to overcome this what is needed is a series of specialists, centralized facilities which would be capable of removing metal from waste water and regenerating or processing the metal loaded sorbent and then converting the recovered metal into reusable form. Alternatively, if the biosorbent used is a waste product, its incineration could be used to produce metal rich slag.

Looking into the economics, feasibility in terms of scale up and working efficiency as a technology the microbial biosorbent provide encouraging results to be utilized in wastewater treatment. What is needed is an extra moral input from the industries generating metal polluted waste water to invest into such clean up technologies before discharging their liquid effluents into the water bodies.

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